THE COLLECTING PERFORMANCE OF HONEY BEES UNDER LABORATORY CONDITIONS [1]

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An investigation by Behling (1929) established that the honey bee, *Apis mellifica,* possesses a "Zeitgedächtnis" or time-memory based on the period of the solar or 24-hour day. As is now well known (for a recent discussion, see Renner, 1960), bees, which have been trained to collect sugar water during specific periods of time on several consecutive days, continue to return to the collecting station with greatest frequency at 24-hour intervals when the sugar solution is no longer present. These results obtain when bees are studied under natural environmental conditions or when the animals are maintained in the laboratory under conditions of constant temperature, light intensity and humidity. They indicate, indeed, that the bee has a timing mechanism or biological clock. The functioning of this mechanism is also demonstrated by the time-compensated orientation of honey bees first described by von Frisch (1950).

Studies by Wahl (1932) and newer work by Renner (1955b, 1957, 1959b) confirmed Behling's findings. These investigations also contributed more information regarding this time-memory, and established that this particular indicator of periodicity does share important characteristics with other long-cycle physiological rhythms. Such rhythms have been described for many species of micro-organisms, plants and animals (for recent reviews, see: Annals of The New York Academy of Sciences, 1962; Cloudsley-Thompson, 1961; Cold Spring Harbor Symposia on Quantitative Biology, 1960).

In the course of such investigations, Renner (1955a) developed techniques which can be used to maintain a colony of honey bees for long periods of time in an essentially normal state in the laboratory. Therefore, the investigator has a means of studying indicators of biological rhythmicity of this species throughout the year under constant conditions of light, temperature and humidity. These laboratories have been described and illustrated in detail (Renner, 1955a, 1959a), and the results of studies conducted in them have been published (Renner, 1955b, 1957).

For long-term observation and experimentation, study of the cycle of the return of trained bees to an empty collection station is severely limited. The amplitude of the cycle falls sharply after three or four days (Wahl, 1932). As a

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consequence, the precise identification of the major phase, the time of the greatest frequency of visits to the collecting station, is no longer possible. If a sugar solution is available at all times to bees kept under constant conditions of light, temperature and humidity, will the workers continue to collect during an extended period of time? Is collecting activity then periodic? Does it also indicate the presence of the timing mechanism in this species? These are the questions which the present study was designed to answer.

Materials and Methods

Observations of a colony of honey bees which had been living in a bee laboratory for three months while being used for other work suggested that workers do collect sugar solution at varying levels during at least a three-week period under conditions of constant light, temperature and humidity. Because this group had begun to act strangely, it was replaced on February 21, 1961, by a colony which had been kept under natural conditions in the garden of The Institute of Zoology, University of Munich. The temperature of the laboratory in which the bees lived was constant at 27° C., its relative humidity fluctuated non-rhythmically between 48% and 52% and the light intensity during the first three months of the study was constant at approximately 1000 lux, as measured at a level one meter from the floor.

From February 22 until 15:00 on March 23, workers from this hive were marked for identification while collecting a two-molar sucrose solution placed at first in an open vessel on a small table located three meters from the hive. Later, the bees collected the solution from a glass container of 800 cc. capacity which was inside an aluminum chamber (see Renner, 1959b, for photographs of this type of collecting chamber) which, in turn, was on the collecting table. The only manner in which the bees could enter or leave the chamber was through a small (7 by 7 mm.) opening in the side of the chamber. At the underside of this opening was a photoelectric cell whose red-colored beam was broken each time a bee entered or left the aluminum container. This served as the basis of the automatic-recording system which was used. This system has also been described in detail (Renner, 1959b).

The counting and printing apparatus was kept in another laboratory, one floor above the bee laboratory. Consequently, the bees could be left undisturbed for long periods of time. During the first month, an investigator did enter the room every day to mark bees, to observe their collecting, to renew the sugar solution and to remove dead bees. However, during the periods of the recording of activity, the only times a person went into the bee room were at irregular intervals, every five to seven days, when fresh sugar solution was placed in the collecting chamber and dead animals were removed. These procedures were accomplished in most cases in roughly two minutes. The times of these interruptions are indicated by x's in the figures.

The recording of activity, *i.e.*, visits of workers to the collecting chamber, was begun at 15:00 on March 23. At this time it was known that 20 to 25 workers were collecting well. The recording was continued with only two breaks, one on April 15 and one on April 19, until 17:45 on May 2 (Period 1). Both these breaks in the record were caused by minor failures of the printing apparatus.

From the late afternoon of May 2 until 10:00 on May 20, no recordings were made. However, during this interval, sugar solution was available, and the conditions of light, temperature and humidity which had obtained since February 21 were maintained with one exception, on May 19, from 8:00 to 16:10, when the bee room was dark while electrical repairs were made. Also during this interval, the laboratory was cleaned, pollen combs were changed and the bees and their hive were checked for disease or any abnormality.

Recording commenced at 10:00 on May 20. Again, roughly 20 bees were known to be collecting the sugar solution. The activity was recorded until 20:00 on June 18 (Period 2). During this time, 37 hours worth of data were lost because of mechanical difficulties. The environmental conditions were those described earlier. On May 30, the room was again dark from 8:30 until 11:15 because of electrical failure. Further, in an attempt to ascertain the pattern of collecting which occurred after exposure to alternating light and dark periods, 12 hours of dark (18:00 to 6:00) alternated with 12 hours of light (6:00 to 18:00) from 18:00 on June 7 through 6:00 on June 9. After this time, light intensity was constant at 1000 lux.

The level of collecting is expressed by the number of impulses recorded per hour. Since, in general, one impulse is recorded when a bee enters the chamber and a second one when she leaves, the number of impulses equals the number of visits × 2. With the exception of daily averages which are given in Figures 1 through 4, all results have been presented in these figures in terms of the number of impulses per hour. No moving means or averages for several hours have been included.

Results

For Period 1, March 23 through May 2, some collecting activity was recorded for each of the 952 hours for which data were complete. The average level of activity for this period was 225.3 impulses/hour. The minimum, recorded in one case, was 3 impulses/hour, and the maximum, also recorded in one instance, was 559 impulses/hour. During Period 1, the average amount of sugar water collected was 2.50 cc./hour. The level of activity for Period 2, May 20 through June 18, was lower than that for the first period. The average activity for the 648 hours for which complete data were available was 139 impulses/hour. During 20 one-hour periods when the laboratory was light, no activity was recorded. In addition, during those times when the laboratory was dark, there was little or no activity at the collecting station. The maximal activity, recorded for one 60-minute period, was 509 impulses, and the average amount of sugar water collected was 2.20 cc./hour.

Although during Period 1 there was a gradual drop-off in average daily activity from the beginning of recording until its termination, during neither period were there sharp and continuous decreases which might indicate an extremely poor condition of the colony. The daily averages, which are given in Figures 1 through 4, did not indicate any periodic fluctuations when considered against the day of the month.

Figures 1 and 2 present the curves of activity for all days of Period 1 except March 23, the day on which the recording was begun. On this day for 17:00, the

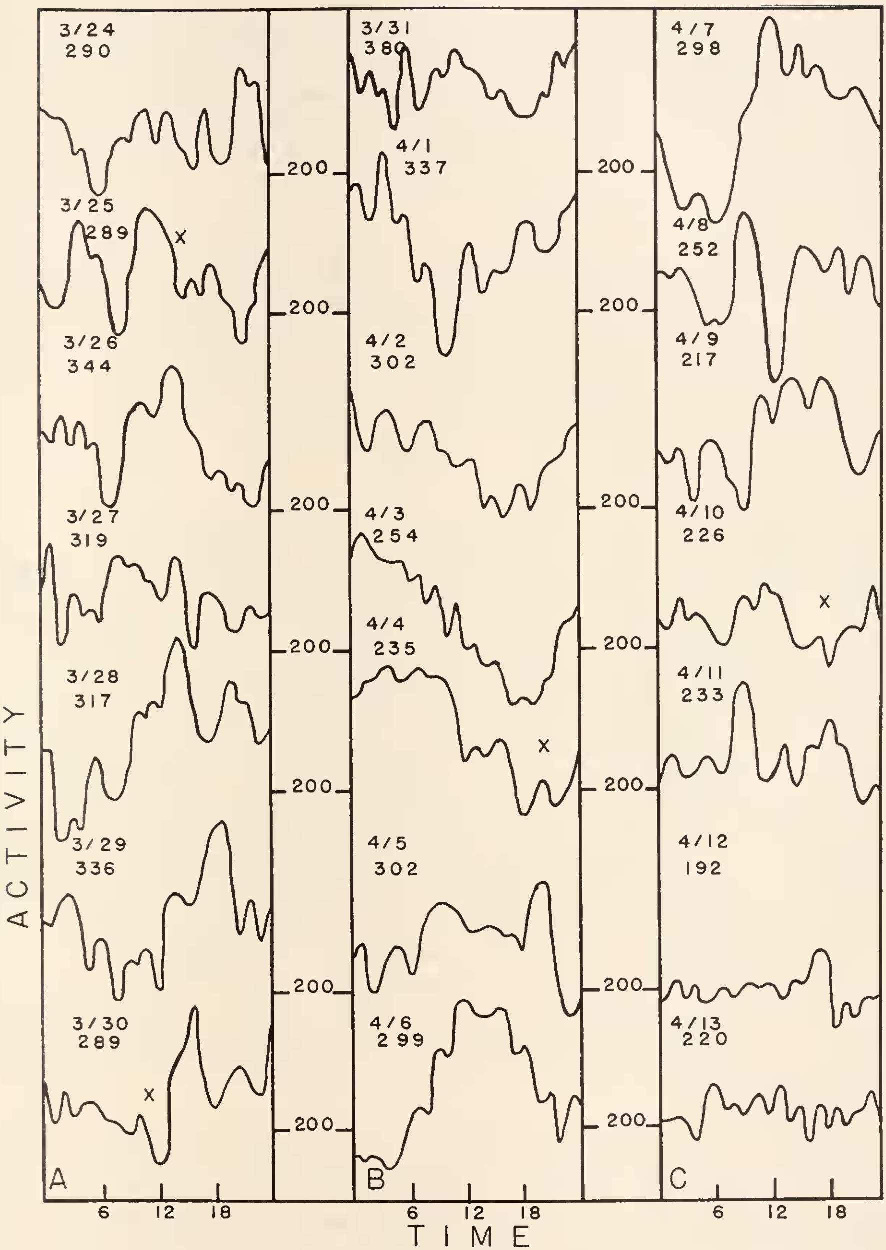

FIGURE 1. The curves of collecting activity for March 24–30 (A), March 31–April 6 (B), and April 7–13 (C). The number of impulses (the 200 level is indicated) per hour is plotted against time in hours, Central European Time. The daily average is given under the respective date. The x's indicate the times at which fresh sucrose solution was placed in the collecting chamber.

first hour for which data were complete, 209 impulses were recorded. Activity then rose to a high of 441 impulses at 21:00 and decreased to 340 impulses at 24:00. During the next 24 hours (Fig. 1, A) activity dropped rather sharply to a low at 6:00, rose again during the middle part of the day, and after lows at 16:00 and 19:00 and a high at 17:00 increased to the maximum at 21:00, after which it decreased. The curve of activity for March 25 was a three-peaked one with collecting having been fairly vigorous at 4:00, over the midday hours and again at midnight. For March 26, the curve was somewhat smoother, and again activity was high during the middle of the day. The picture was generally the same on March 27 and on March 28 when a definite peak for the 24-hour period was recorded at 14:00. Maximal activity on March 29 occurred at 19:00 while generally low activity characterized the late morning and midday hours. This situation was also seen on March 30; however, on that day the peak of collecting occurred at 16:00. The shape of the curve for March 31 (Fig. 1, B) was roughly similar to those for March 26, 27, and 28, except that on this day, the highs at midday and midnight were of the same level, and activity for 6:00 was slightly higher than that at 11:00.

On April 1, a new relationship between the times of maximal and minimal collecting and hours of the solar day was seen. Activity was greatest at 4:00, dropped sharply to a low at 10:00, and then increased fairly smoothly through midnight. The curve for April 2 approaches a sinusoidal one, as do those for April 3 and 4. On April 5, the abrupt increase after 18:00 interrupted the tendency for collecting to fall gradually from the high at 9:00. The curves for April 6 and 7 (Fig. 1, B and C) are among the smoothest recorded during Period 1. In both cases, the maximal activity occurred at noon. That for April 8 resembled the curve for April 7 except that clearly minimal levels of activity at 12:00 and 13:00 were interposed between the late morning and early afternoon periods of greater activity. Again on April 9 collecting was vigorous during the midday with the activity continuing at a high level until 17:00. On April 10, the level of collecting was maximal at 11:00, but the amplitude of this curve is not great. At 9:00 and again at 18:00 on April 11 activity was high, and for April 12 only the maximum at 17:00 and the minimum at 19:00 interrupted an essentially straight line relationship between time and the levels of collecting.

On April 13 and 14 (Fig. 2, A), the amplitudes of the curves were low, while the form of the curve for the 14th was somewhat similar to those for April 2 through 4. For April 15 through 22 (Fig. 2, A and B), collecting was generally lower during the midday hours than it was during the early morning and late evening periods.

Basically, the curves of activity for April 23 through 25 resemble those of April 2 through 4. On April 26 (Fig. 2, C), activity was clearly maximal at 5:00 and was again high at 15:00. During the following solar period, the bees collected relatively actively from 6:00 through 14:00, after which hour the level fell off steeply to a minimum at 18:00. The curve for April 28 is two-peaked with highs at 7:00 and 18:00 and with lows at 1:00 and 13:00. From April 29 through May 1, the temporal patterns of collecting were similar to one another; the daily maxima occurred in each case in the morning hours (6:00 to 8:00), and the daily minima were recorded just after midnight (1:00 to 2:00). On

April 30 and May 1, minor peaks were seen in the afternoon, and for May 2, the data which were available indicated a maximum at 13:00.

The mean period lengths were found for three different blocks of consecutive days (seven to nine days) where some repetition of pattern could be seen. For

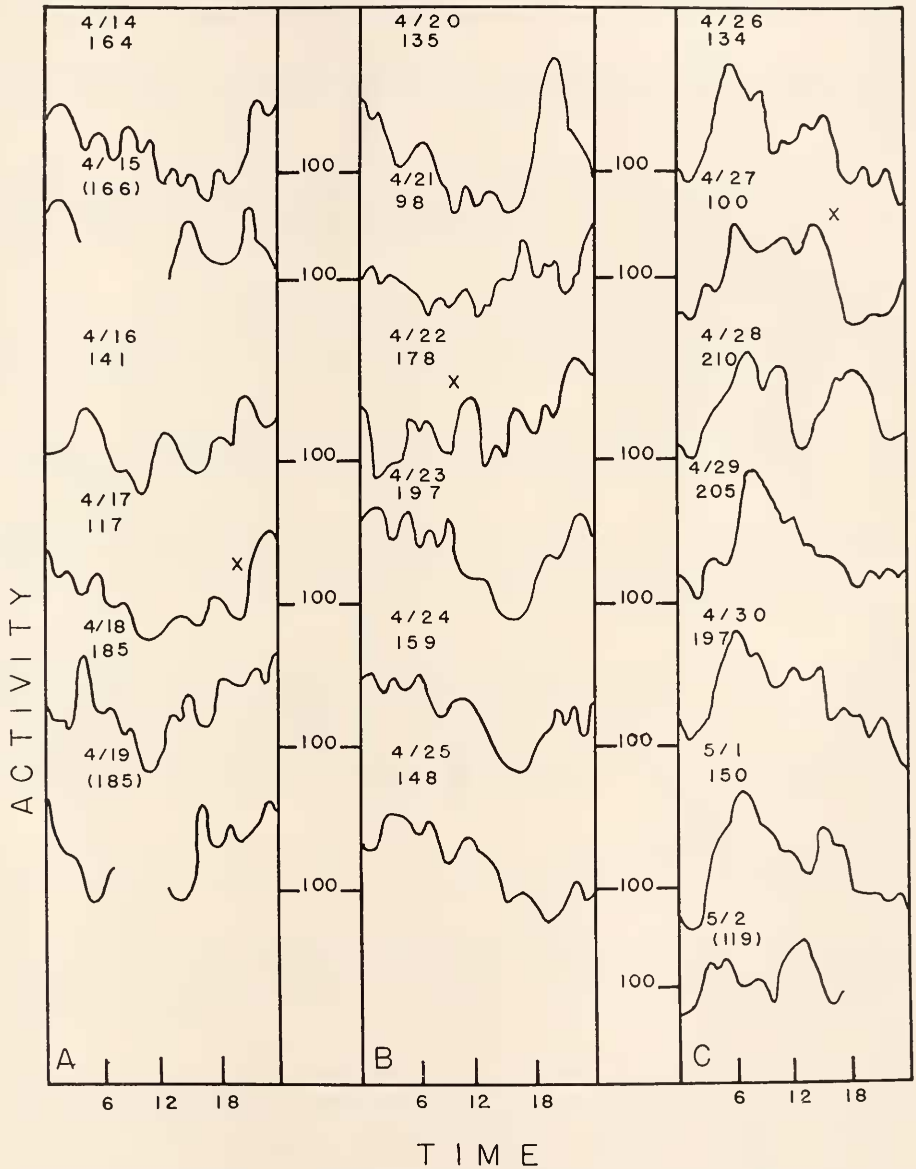

FIGURE 2. The curves of collecting activity for April 14–19 (A), April 20–25 (B), and April 26–May 2 (C). Here the 100 level of impulses is indicated. Remainder of the legend as in Figure 1.

March 25 through 31, the mean interval between highs occurring between 11:00 and 19:00 was 24.0 ± 3.2 hours (range, 19–29); for April 14 through 22, using maxima which fell between 20:00 and 24:00, it was 24.0 ± 2.12 (range, 21–28); and for April 23 through May 1, using maxima which fell between 2:00 and 8:00, the mean was 24.0 ± 1.66 hours (range, 21–26). When the lengths of the periods for these three blocks of days were considered together, the mean was 24.0 ± 2.4 hours.

For single solar day periods, several different forms were described in terms of the level of collecting relative to hours of the day during Period 1. These were:

(1) Relatively high levels of activity occurred bridging midday and midnight periods with intervals of lower levels interposed between them. March 24–27 (Fig. 1, A), March 31 (Fig. 1, B), April 9 (Fig. 1, C), and April 27 (Fig. 2, C).
(2) Collecting was clearly maximal during the midday period. March 28 (Fig. 1, A), and April 6 (Fig. 1, B) and 7 (Fig. 1, C).
(3) Collecting was clearly maximal within a few hours of midnight. This is the inverse of Pattern 2. April 1 (Fig. 1, B), April 17, 18, 19, and 20 (Fig. 2, A and B).
(4) Collecting was high between 0:00 and 12:00, decreased from 12:00, and rose again. April 2 (Fig. 1, B), 3 and 4, and April 23 through 25 (Fig. 2, B).
(5) Collecting activity was maximal roughly midway between 0:00 and 12:00, and was minimal around the midnight hour. April 26, and April 29 through May 1 (Fig. 2, C).

Figures 3 and 4 illustrate the collecting activity for all days of Period 2 except May 20, the day on which recording commenced, and June 12, a day for which no data were available because of mechanical difficulties. As has been pointed out, the laboratory was dark from 8:00 through 16:10 on May 19, the day before recording started at 10:00. From the start until 15:00 on May 20, no collecting occurred; after 15:00 it increased gradually through 24:00, for which hour 157 impulses were registered.

As can be seen in Figure 3, A, on May 21, the level of collecting decreased after midnight, and from 9:00 to 12:00, none was recorded. The activity then increased and remained at a level similar to that of the preceding midnight through the remainder of the day. Again on May 22, the activity of the animals at the collecting station decreased during the early morning hours, virtually ceased from 6:00 until 10:00, increased after 10:00, and was maximal between 14:00 and 16:00. A minor peak was seen at 21:00. The pattern for May 23 was fairly similar to that of May 22 with the exceptions that activity ceased from 4:00 until 8:00; the maximum occurred at 14:00, and the minor peak of the evening was seen at 20:00. The morning hours of May 24 were characterized by a steady increase in activity to the peak at 11:00, while those of the remainder of the day showed decreasing levels of collecting. For May 25, activity increased during the morning hours with highs at 6:00 and from 9:00 to 11:00. During the post-noon period a high at 15:00 and a low at 19:00 were obvious.

The data for May 26 and 27 (Figure 3, B) continued to indicate those tendencies typical of the preceding days. Those for May 27 trace out a fairly smooth curve. The highest point on this day occurred at 10:00 and the lowest at 18:00. On May 28, collecting was low during the entire 24-hour period, and at most, it can be said that the bees were more active from 0:00 through 11:00 than they

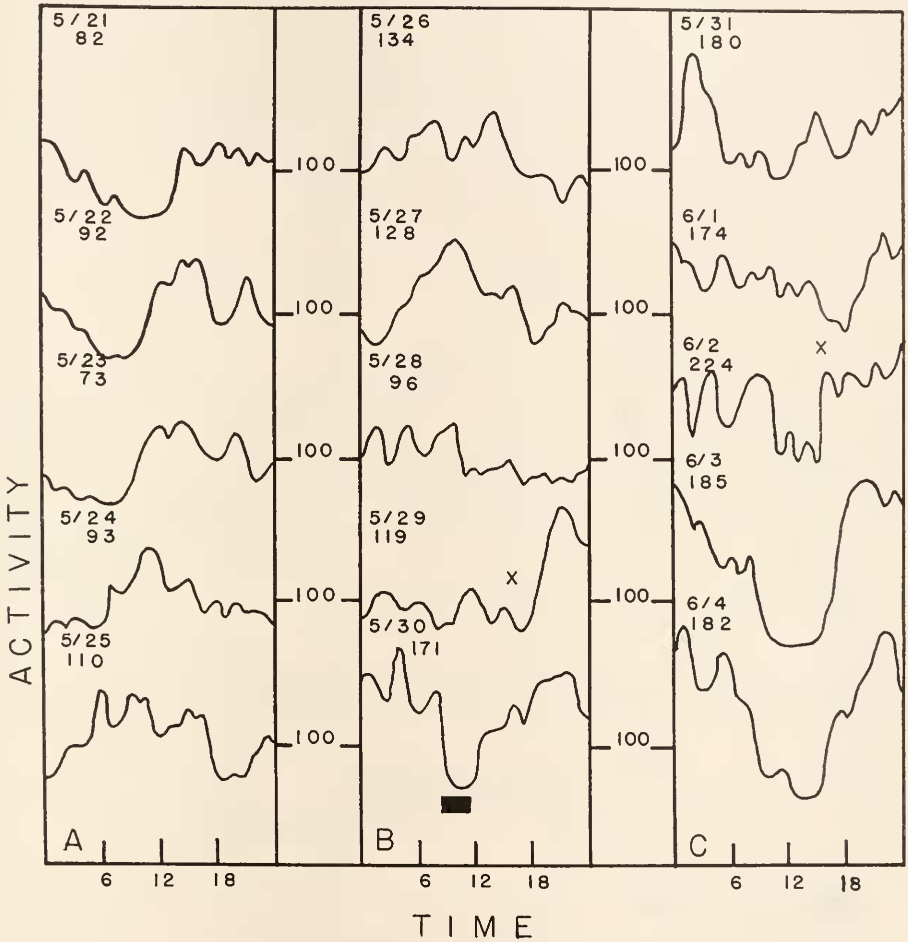

FIGURE 3. The curves of collecting activity for May 21–25 (A), May 26–30 (B), and May 31–June 4 (C). The black band indicates when the laboratory was dark. Remainder of the legend as in Figures 1 and 2.

were during the remainder of the day. The low activity continued through 17:00 on May 29. Collecting then increased rapidly to the peak at 21:00. From then until 10:00 on May 30 the levels of activity dropped fairly regularly. It should be noted that the laboratory was again dark from 8:15 until 11:30 on this day, and after this time, collecting increased until 22:00 after which it began to decrease.

In general, solar periods characterized by lower activity during midday and greater collecting during early morning and late afternoon and evening hours were seen from May 31 through June 6 (Figs. 3, C and 4, A). The curves for June 3 and 4 were especially smooth. A similar picture was indicated by the data for the first 18 hours of June 7. On this day, as indicated in Figure 4, A, the laboratory was darkened immediately after 18:00. After 6:00 on June 8, at which time the light intensity was again 1000 lux, the bees began to collect. The level of their activity was highest at 14:00 and immediately after that time began to decrease. The laboratory was dark between 18:00 on June 8 and 6:00 on June 9. After this time, light was restored and was then kept constant for the rest of the period of recording. It is important to note that for June 9, the form of the curve is approximately the same as that for June 8 even though, as has been emphasized, the laboratory was not darkened at 18:00.

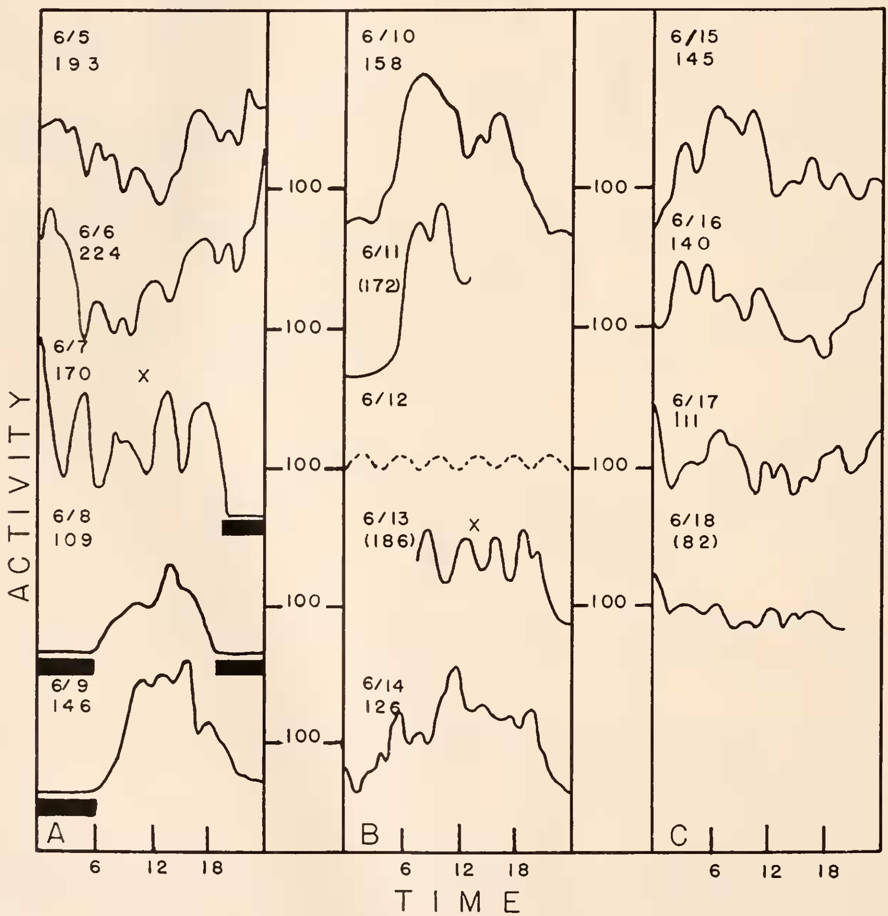

FIGURE 4. The curves of activity for June 5–9 (A), June 10–14 (B), and June 15–18 (C). Remainder of the legend as in Figures 1–3.

This similarity was seen again on June 10 (Fig. 4, B), although the increase in activity started earlier on June 10 than on June 8 and 9, and the results for the periods from 8:00 through 16:00 vary. Unfortunately, no data were available after 14:00 on June 11 until 8:00 on June 13. However, those which were recorded for the first 14 hours of June 11 indicate a pattern which is directly comparable with that for the same hours on June 10. In addition, those data for June 13 after 8:00 showed the tendency for activity to be fairly high until 20:00, after which it fell rather steeply. The curve for June 14 is also very similar to those for June 8 through 10, and in this case the daily maximum occurred at 12:00 and the minimum at 24:00.

Greater activity occurred earlier, between 6:00 and 11:00 on June 15 (Fig. 4, C), than on June 14, and a low was seen immediately after midnight. The period of more vigorous collecting was during the morning hours on June 16. The minimum for this day occurred at 18:00, after which time activity increased. For both June 17 and 18, the curves are of low amplitude. Activity was not great on these days, and only a tendency for midnight collecting to be greater than that of midday was seen.

From the results of Period 2, two blocks of consecutive days were used to calculate mean period lengths. For May 21 through 27 (seven days), by using highs between 10:00 and 18:00, the mean was 23.3 ± 2.60 hours (range 20–27), and for May 31 through June 7 (eight days), by using highs recorded between 0:00 and 4:00, it was 23.7 ± 2.40 (range, 20–28). When these periods are considered together, the mean period length is 23.5 ± 2.45 hours. When these periods from recording Period 1 and from recording Period 2 are used, mean period is 23.8 ± 2.40 hours.

As during Period 1, different forms were seen, but here the variation was less than during the first period, and two principal patterns were obvious.

(1) Collecting was maximal during midday. May 23 through 27 (Fig. 3, A and B) and June 8 through 14 (Fig. 4, A and B). This is comparable to Pattern 2 of Period 1.
(2) Collecting was maximal within a few hours of midnight. May 31 through June 7 (Figs. 3, C and 4, A). This is an approximate inverse of Pattern 1, and is comparable to Pattern 3 of Period 1.

Also to be noted are possible effects of the changing of the sugar solution and the interruption made necessary by this on subsequent activity of the bees. The times when the changes were made are in all cases indicated in the figures. On March 25, the solution was changed shortly after 15:00, and during the next 72 hours, generally high activity occurred at 14:00. A high on March 31 at 11:00 was about 24 hours after the interruption of March 30. The high of April 5 at 20:00 was again approximately 24 hours after the solution was changed on April 4. On April 10, a new cylinder of sucrose solution was presented just after 17:00; on April 11, activity was high at 18:00 and on the following day at 17:00. However, no obvious relationships were evident between the interruption on April 17 or that on April 22 and events on the days which followed. The last change during Period 1 was made shortly after 16:00 on April 27. A peak of activity occurred at 18:00 on April 28.

The first change during Period 2 was made just before 16:00 on May 29. On May 30 at 16:00 and on May 31 at 15:00 minor increases in collecting levels were seen. Nothing comparable was seen on June 3 and 4, the days following the change on June 2 before 15:00. An interruption occurred again after 11:00 on June 7. During the following two solar periods only very minor decreases in the levels of collecting were seen between 11:00 and 12:00. The last change occurred shortly after 13:00 on June 13. A clear maximum was seen at 12:00 on June 14.

Discussion

The results of the present work show clearly that a colony of honey bees, maintained under laboratory conditions of constant light intensity, humidity, temperature and provided with a constant supply of sugar solution, does lend itself to long-term observation and experimentation. Since an investigator need enter the bee laboratory only briefly, at most every five or six days, to renew food supplies and to check the colony and its laboratory, any effects of these manipulations can be kept minimal. During the progress of this study, there was some decrease in the average level of collecting activity. However, this decrease was not constant and is not believed to have been indicative of a poor condition of the colony. This observation was supported by checking the contents of the hive and by keeping a record of the number of individuals that died. The changes in the average daily activity may be explained, in part, by changes in the numbers of active workers.

There was no regular or cyclic variation in these changes. Since data were available for only slightly more than a two-month period, no definite statement concerning the presence or absence of seasonal variations can be made. It is possible that records for a longer period of time would reveal correlations between levels of collecting and seasonal changes. Such correlations have been found for the levels of metabolism of the potato (reviewed by Brown, 1960) and for the metabolic cycles of the fiddler crab (Webb and Brown, 1961). These organisms were also maintained under constant conditions in the laboratory. A colony of honey bees does provide us with a population whose activity should be observed for a year or more under constant conditions.

Not only did the level of collecting of the bees vary from day to day during the two periods of observation, but also, the levels from hour to hour varied. In many cases, the variations were systematic within one solar period, and similar patterns of these variations were maintained from two to nine consecutive days. The mean frequency of these cycles, calculated from five different blocks of days, was 23.8 $\pm$ 2.40 hours. These findings suggest that the solar-day timing mechanism known to exist in honey bees is involved in their long-term collecting performance. The reviews of Aschoff (1960) and Pittendrigh (1960) present characteristics of so-called circadian rhythms and theories regarding their organization. Both these authors hold that circadian rhythms are those whose periods under constant conditions *approximate* the period of the earth's rotation, and that these rhythms are endogenous to the living system. The latter author emphasizes the precision of the periods of these cycles during a sequence of days, and states, "Observed standard errors of the period may be less than 2 minutes per day" (Pittendrigh, 1960, Table I, p. 160). Such precision was not manifest in the cycles of the

bees. Perhaps a system of recording from which activity per minute or five-minute intervals was available would serve to sharpen these cycles of collecting.

During several solar periods, the differences in the levels of activity were not great and/or varied in a seemingly random manner. Further, in many cases the forms of the cycles changed abruptly from day to day. Therefore, it is illustrated once again that the investigator of biological timing is not confronted with simple cycles to be analyzed solely in terms of period-length. The radical changes seen in the temporal performance of the bees suggest immediately comparisons with results of a series of studies by Brown and his co-workers (see Brown, 1960, for a review of this work). These investigators have described cases in which form, and therefore the period, of cycles of indicator processes do change. At times, these changes are abrupt and irregular from day to day, even when the organisms are maintained under constant conditions including atmospheric pressure. Undoubtedly important to the understanding and elucidation of the mechanism of biological rhythmicity is that aspects of these organismic changes have been correlated with non-cyclic changes which are superimposed on statistically rhythmic changes in the levels of several environmental factors, *e.g.*, cosmic radiation, barometric pressure. Such analyses have not been made with the present data. However, seen here were inversions of the form of the cycles, a phenomenon seen often in the results from Brown's laboratory (Brown, 1957; Terracini and Brown, 1962).

As is well known and is accepted by all students of biological clocks, under so-called constant conditions, the phases of circadian cycles do shift gradually relative to solar time, since the frequency may not be precisely 24 hours (Aschoff, 1960; Brown, 1960; Pittendrigh, 1960). This characteristic has been used by many investigators as proof for the endogenous nature of such cycles (Aschoff, 1960; Pittendrigh, 1960), while it has been explained by another by the theory of autophasing (Brown, 1959, 1960, 1962c). The rate at which such shifts occur, or the length of the free-running period, has been correlated with the constant light intensity to which the organisms are exposed and the habit (diurnal or nocturnal) of the animals in their natural environments (Aschoff, 1960; Pittendrigh, 1960). The performance of the bees does not resemble strictly such changes, for the phases of their activity did not move regularly to a later or earlier time of the solar day or with a constant rate. Rather, often, the relationships of particular phases of activity with solar time changed abruptly and at irregular intervals and in different directions in time. Here it should be noted, as Renner (1955a) found, it is necessary to maintain a very high light intensity (1000 to 1200 lux) in a bee laboratory in order that the bees fly actively. Most forms whose persistent rhythms have been studied have been kept in the dark or at substantially lower light intensities (see Aschoff, 1960, for examples).

Another characteristic of persistent cycles which should be considered here is the temporal lability of phases. Phases can be set relative to specific real times by so-called perturbations of which light-dark and temperature changes seem to be the most effective (Pittendrigh, 1960). Studies of the time-memory of the bee have proved such lability characteristic of this cycle. Phases of vigorous collecting can be set by training at different times or during several different periods of the solar day (Behling, 1929). Exposure to 4.5° C. delays the time of collecting and

therefore shifts the phases (Renner, 1957). During Period 1 of the present study, no changes in temperature or light intensity occurred in the laboratory. The effects of change of light during Period 2 will be discussed later. During both periods of recording, the sugar solution was changed at irregular intervals. The observations suggest strongly that the effects of these changes were not extremely great, were not constant, and were ephemeral.

What is the explanation for the saltatory phenomena which have been observed? It is extremely important to point out that in the present work, a *population* of animals was observed. This group is one which has evolved a complex social life that includes a system of communication. This system serves, among other functions, the recruiting of new workers for food collecting (von Frisch, 1946, 1948). One must consider again that individual workers can be trained to forage at particular times, during one or several intervals per solar day, and tend then to return to the collecting station at 24-hour intervals as set by the time of training (Behling, 1929). The data recorded for each day of the present study represent the summation or a complex of the collecting activity of 20 to 30 bees. Is it not probable that the individuals' cycles were not synchronized? Also very probable was that phases of each cycle had been set by a bee's first finding and collecting the sucrose solution, by chance or as a result of having been recruited by active workers. These considerations can explain the different daily patterns which have been seen. On a day when a peak of activity occurred at midday, many of the active workers or the dominant ones were those whose period of most intense collecting had been set at noon. During a day when two periods of high activity were seen, there were either two groups of dominant collectors whose phasing varied or one group which was collecting intensely during two different periods. The maintenance of a pattern during consecutive days, as well as the abrupt changes in form, can be explained, as follows. Workers, whose phases had been set as suggested, were the dominant individuals at the collecting station for several days, and the pattern was repeated. As these died, others whose phasing was different became dominant, and the form of the summated cycle changed. In addition, the possibility that workers were shifting their own cycles, which will be discussed later, must be considered here.

The results of Period 2 certainly illustrate that daily cycles were sharpened by changes in the light intensity in the laboratory. In all probability this increase in precision was the result of the synchronization of the cycles of the collectors. However, fairly regular cycles were not repeated for more than eight days. The high light intensity to which bees must be exposed and its possible effect on the cycles must be considered again as appropriate suggestions for the explanation of the shifts and warpings of the cycles. Possible changes in the dominant workers also can help explain this loss of precision. In addition, it is likely that a worker's phasing is shifted by the changes in light intensity she meets as she flies from a dark hive through an area where light intensity is very high, and into a dark collecting chamber. A glass-walled hive and collecting cylinder might improve this situation. In addition, individuals' performances must be observed during long periods of time to see what the contribution of each to the summated cycle is. A recent paper by Stephens (1962) shows how important it is to consider the

contributions of individual fiddler crabs to a rhythm of color change of the population.

Lastly, it is possible that some aspects of the collecting activity of the bees were mediated by external factors whose changes in intensity, rhythmic or not, constitute stimuli for the honey bee. The field study of Renner (1959b) proved that an environmental factor, the position of the sun, when changed by rapid transport of the bees, does warp the form of the cycle of collecting by trained bees. Other possibly effective factors have been considered in two other discussions (Renner, 1960, 1961). It has become increasingly obvious that receptor capacities, and therefore the factors to which organisms can respond, are not known or understood fully. These capacities and reactions may be of utmost importance for the functioning of the organismic time piece and for the future understanding of its mechanism (Brown, 1962a, 1962b, 1962c; Brown, Bennett and Ralph, 1955; Brown, Bennett and Webb, 1960; Webb, Brown and Brett, 1959).

Summary

1. The collecting activity of honey bees, *Apis mellifica,* maintained in a bee laboratory, was recorded automatically through two different periods, the first of 40 consecutive days, and the second of 30 consecutive days. During both these periods, a two-molar sucrose solution was available at all times, and the temperature and humidity were constant. During the first period of recording, the light intensity was also constant at approximately 1000 lux. This was true also of the second period with the exception of four times when the laboratory was dark. Under these conditions, workers continued to collect the sugar solution, and the colony remained in good condition. Therefore, such a population is favorable for long-term observation and experimentation.

2. During both these periods of recording, the average daily collecting activity varied from day to day; however, these variations were not regular or cyclic. The period or frequency of cycles described in terms of hourly levels of activity and solar time was found to vary from 19 to 29 hours, and the mean value was 23.8 ± 2.40 hours. Several different forms were described by these parameters, and changes from one to another were not always gradual, but were saltatory in nature. Changes in light intensity did effect greater precision of the cycles, probably by synchronizing the rhythms of individual workers.

3. The results are discussed in terms of biological rhythmicity, and possible explanations for the saltatory nature of the daily cycles are presented.

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